REMARKS

Applicants have amended the specification to incorporate sequence identifiers and correct typographical errors. Applicants have amended the sequence listing to include sequence identifiers for the sequences on pages 31-33. No new matter has been added.

Attached is a marked-up version of the changes being made by the current amendment.

Applicant asks that all claims be examined. Payment is enclosed for the Petition for Extension of Time and declaration surcharge fees. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: 10/4/02

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Version with markings to show changes made

In the specification:

Paragraph beginning at page 30, line 35, has been amended as follows:

A part of *PRKAG3* including codon 41 was amplified in 10 μl reactions containing 100 ng genomic DNA, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 4.0 pmol of both forward (AMPKG3F3:5' – GGAGCAAATGTGCAGACAAG-3') (SEQ ID NO:33) and reverse (AMPKG3R2:5' -CCCACGAAGCTCTGCTTCTT-3') (SEQ ID NO:34) primer, 10% DMSO, 1 U of *Taq* DNA polymerase and reaction buffer (ADVANCED BIOTECH, London, UK). The cycling conditions included an initial incubation at 94° for 5 min followed by 3 cycles at 94°C (1 min), 57°C (1 min) and 72°C (1 min), and 35 cycles of 94°C (20 sec), 55°C (30 sec) and 72°C (30 sec). Allele discrimination at nucleotide position 122 was done using the oligonucleotide ligation assay (OLA, LANDEGREN, *et al.*, Science, 241, 1077-1080, 1988). The OLA method was carried out as a gel-based assay. Each 10 μl OLA reaction contained 0.5 pmol of each probe SNPRN-A (5'Hex-TGGCCAACGGCGTCCA-3') (SEQ ID NO:35), SNPRN-G (5'ROX-GGCCAACGGCGTCCG-3') (SEQ ID NO:36) and SNRPN-Common (5'phosphate-AGCGGCACCTTTGTGAAAAAAAAAA-3') (SEQ ID NO:37), 1.5 U of thermostable AMPLIGASE and reaction buffer (EPICENTRE TECHNOLOGIES, Madison, WI) and 0.5 μl of the AMPKG3F3/AMPKG3R2 PCR product. After an initial incubation at 95°C for 5 min, the following thermocycling profile was repeated 10 times: denaturation at 95°C (30 sec), and probe annealing and ligation at 55°C (90 sec). After OLA cycling, 1 μl of product was heat denatured at 94°C (3 min), cooled on ice, and loaded onto 6% polyacrylamide denaturing gel for electrophoresis on an ABI377 DNA sequencer (PERKIN ELMER, Foster City, USA). The resulting fragment lengths and peak fluorescence were [analysed] analyzed using GENESCAN software (PERKIN ELMER, Foster City, USA).

Paragraph beginning at page 32, line 5, has been amended as follows:

A microsatellite 127B1 (*MS127B1*) was cloned from BAC 127G7 containing pig *PRKAG3*. The BAC clone was digested with *Sau*3AI and the restriction fragments subcloned

into the *Bam*HI site of pUC18. The resulting library was probed with a $(CA)_{15}$ oligonucleotide probe [labelled] labeled with [γ-32P]-dATP. Strongly [hybridising] hybridizing clones were sequenced and primers for PCR amplification of microsatellite loci were designed. Ten μl PCR reactions were performed containing 100 ng genomic DNA, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 4.0 pmol of both forward (MS127B1F:5' -Fluorescein-CAAACTCTTCTAGGCGTGT-3') (SEQ ID NO:38) and reverse (MS127B1R:5' -GTTTCTGGAACTTCCATATGCCATGG-3') (SEQ ID NO:39) primers, and 1 U of *Taq* DNA polymerase and reaction buffer (ADVANCED BIOTECH, London, UK). The cycling conditions included an initial incubation at 94°C for 5 min followed by 3 cycles at 94°C (1 min), 57°C (1 min) and 72°C (1 min), and 35 cycles of 94°C (20 sec), 55°C (30 sec) and 72°C (30 sec). The PCR products (0.3 μl) were separated using 4% polyacrylamide denaturing gel electrophoresis on an ABI377 DNA sequencer (PERKIN ELMER, Foster City, USA). The resulting fragment lengths were [analysed] analyzed using the GENESCAN and GENOTYPER software (PERKIN ELMER, Foster City, USA).

Paragraph beginning at page 33, line 13, has been amended as follows:

Sequence of primers used to amplify the *RN* mutation region:

RNU: 5' GGGAACGATTCACCCTCAAC 3' (SEQ ID NO:40)

RNL: 5' AGCCCCTCCTCACCCACGAA 3' (SEQ ID NO:41)

Paragraph beginning at page 33, line 24, has been amended as follows:

The sequence of the RNL modified primer including a control tail with a *Bsr*BI site is:

RNLBsrA14: 5' $A_5C_2A_7$CCGCTCAGCCCCTCCTCACCCACGAA 3' (SEQ ID NO:42)